Investigation into the Natural History and Metabolic and Molecular Basis of RASopathies

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Abstract:
The RASopathies are a group of developmental disorders caused by genetic changes in the genes that compose the Ras/mitogen activated protein kinase (MAPK) pathway. New RASopathies are being diagnosed frequently. This pathway is essential in the regulation of the cell cycle and the determination of cell function. Thus, appropriate function of this pathway is critical to normal development. Each syndrome in this group of disorders has unique phenotypic features, but there are many overlapping features including facial features, heart defects, cutaneous abnormalities, cognitive delays, and a predisposition to malignancies. This research study proposes to collect and store human bio-specimens from patients with suspected or diagnosed RASopathies. Once obtained, blood and/or tissue samples will be processed for: metabolic function studies, biomarkers, genetic studies, and/or the establishment of immortalized cell lines. In addition, data from the medical record (including neuropsychological evaluations) and surveys will be stored to create a longitudinal database for research conducted at CCHMC or at other research institutions.

Purpose of the Research Project:
Patients who are being evaluated for a RASopathy may have overlapping features, but the disorders individually can be exceedingly rare and many are not yet well characterized. Additionally, available clinical testing is not always diagnostic in this group of patients. We propose to study disorders across the RAS/MAPK pathway, identifying both commonalities and differences, under one unified ongoing research protocol. We propose:

1) To investigate the metabolic and molecular basis of established and suspected RASopathies.

2) Collect specimens derived from blood, buccal cells, sputum, urine, bone marrow, tumor tissue and residual specimens, including but not limited to pleural fluid, ascetic fluid, chyle, skin, lung, lymphatic or renal tissue and/or bronchoalveolar lavage fluid, tissue specimens, and/or cells that are left over from clinical procedures from enrolled patients for research purposes only.

3) Non-invasive or minimally invasive procedures to collect tissues for research purposes only, such as saliva, skin, or blood samples are also allowed. The

collection of all samples from minor subjects will be done only if it is safe for the participant. Clinical studies will take precedence over research procedures.

4) Collect demographic information, medical history, and clinical test results to create a longitudinal research database of participants with suspected or diagnosed RASopathies. Participants will also complete surveys to be included in the research database (see "Research Database" section for details).

5) Provide a facility for long-term storage of bio-specimens and clinical data from participants with suspected or diagnosed RASopathies and their unaffected relatives.

Overall Study Design Summary:

Samples will be stored and tracked by the Cincinnati Biobank team. Demographic information, test results, and medical records will be stored in a REDCap database and maintained by Dr. Weaver's study team. The study team will also maintain a key to track samples and data back to the participant's identity. However, data and samples from the repository will be released in a de-identified state to researchers. Research procedures will not be conducted on the samples or the available data unless the research question is related to RASopathies, the project is approved by the Repository Use Committee, and IRB approval is obtained. Additional detail as below.

Significance of the Study in Relation to Human Health:

The RASopathies are a group of developmental disorders caused by genetic changes in the genes that compose the Ras/mitogen activated protein kinase (MAPK) pathway. This pathway is essential in the regulation of the cell cycle and the determination of cell fate and differentiation. Thus, appropriate function of this pathway is critical to normal development. Each syndrome in this group of disorders has unique phenotypic features, but there are many overlapping features including facial features, heart defects, cutaneous abnormalities, cognitive delays, and a predisposition to malignancies (Tidyman, 2009; Schubbert 2007; Smpokou 2015; Tidyman, 2016).

Individually, each of these syndromes are rare. Together, they are one of the most common groups of genetic conditions in the world. Currently, there is no cure or treatment for the underlying cause of the disease. RASopathies are treated by addressing the manifestations of disease as they appear while also monitoring for progression of symptoms.

We aim to develop a tissue repository and longitudinal database that will aid in the development of new diagnostic tools and treatment strategies for patients with suspected or diagnosed RASopathies. Results from this research may also have implications for those affected by cancer due to the Ras/MAPK pathway's role in the development of malignancies.

Previous Work Done in this Area:

Neurobehavioral and Psychometric Assessments

Alfieri et al (2014) used the Child Behavior Checklist (CBCL), Social Communication Questionnaire version lifetime (SCQ-L), and Modified Checklist for Autism in Toddlers (M-CHAT) to characterize the neurobehavioral features of patients with RASopathies. We have experience with evaluation of the PANESS test, abbreviated IQ test, CogState, and Parents Questionnaires including BRIEF, CBCL, SRS, Conners-3, and the Y-BOCS. They found that mutations in the RAS-MAPK pathway mark an increased psychopathological risk and highlight that autistic-like behavior could be underdiagnosed in patients with RASopathies (Alfieri, 2014).

Results from a 2016 study "indicate that children with CFC syndrome, another RASopathy, are at heightened risk for psychopathology, with attention problems, social difficulties, and unusual behaviors (e.g., obsessive thoughts, strange behaviors, repetitive acts) found to be especially prevalent. With regard to the impact of child neurocognitive and behavioral issues on the caregiving experience, parent self-reported stress was significantly higher among parents of children who engaged in more problem behaviors, and lower among parents whose children could communicate effectively with others. This highlights the need for educational and treatment interventions aimed at addressing sensory needs, increasing functional communication, and identifying and managing challenging behaviors (Pierpont 2016)."

Using the Collaborative Programme for Excellence in Autism criteria, Garg et al (2017) found that 12 of 40 (30%) children with Noonan syndrome had autism spectrum disorder (ASD), 12 of 40 (30%) had some symptoms of ASD, and the remaining 40% had no symptoms of ASD. In the Noonan syndrome ASD group, there was a 5:1 male predominance. Nineteen of 40 (48%) in the Noonan syndrome group and 8 of 9 (88.9%) in the CFC group met clinical criteria for ADHD (Garg 2017).

Genetic Research

Approximately 20-30% of the causative genes behind Noonan syndrome and similar disorders are unidentified. Novel gene variants in *RIT1, RRAS, RASA2, A2ML1, SOS2,* and *LZTR1* were shown to be associated with RASopathies, malignancies, and cardiovascular abnormalities. Additional studies were recommended to characterize the associated phenotypes and functional role of these genes (Aoki, 2016). Cizmarova et al (2016) used direct sequencing and next-generation sequencing of all the known RASopathy genes for 51 patients with typical features of RASopathy syndromes. They found 35 mutations, two of which were novel and potentially pathogenic (one each in *BRAF* and *MAP2K1*). Mutations were observed in 68.63% of cases (35/51).

Phenotypic Research

The prenatal and neonatal findings are less widely studied than the postnatal effects of this group of disorders. Perinatal findings in Noonan, Costello, and CFC are overlapping: polyhydramnios, prematurity, lymphatic dysplasia, macrosomia, relative macrocephaly, respiratory distress, hypotonia, and cardiac and renal anomalies. Fetal arrhythmia and neonatal hypoglycemia are specific for Costello. When there is a normal karyotype and ultrasound findings as above, Noonan, Costello, and CFC should be

considered. Recognition of common perinatal findings of a RASopathy should facilitate perinatal and neonatal diagnosis (Myers 2014).

Neurogenic tumors and hypertrophic neuropathy are unusual complications of Noonan syndrome with multiple letigines and may be an under-recognized manifestation that would warrant surveillance; this observation may have implications for other disorders in their pathway (Conboy 2015).

In the first study to characterize body composition in individuals with RASopathies, researchers collected growth parameters, body compositions, and nutrition for both children and adults with RASopathies. They found that both adipose tissue and muscle mass are compromised in individuals with RASopathies. They called for studies using dual-energy X-ray absorptiometry or imaging studies to more precisely delineate fat and non-fat mass to confirm their findings, which could have an impact on clinical management (da Silva 2016).

Tamburrino et al in 2015 studied 88 individuals with a molecularly confirmed RASopathy. Thirty-three showed growth hormone deficiency and were treated with growth hormone for an average of 6.8 years. Long-term growth hormone therapy, starting in early childhood, resulted in a positive height response compared to untreated patients. During growth hormone treatment, no significant change in bone age velocity, body proportions, or cardiovascular function was observed (Tamburrino 2015).

Researchers in several studies have hypothesized that there is a role for the RAS pathway in regulating bone metabolism due to the short stature and skeletal dysmorphisms that are almost constant in patients with Noonan syndrome. Baldassarre et al found that phalangeal quantitative ultrasound measurements indicated that bone impairment persists in nearly 15% of their cohort with Noonan syndrome, even after correcting for growth retardation, delayed bone age, retarded puberty, and reduced body mass index. They concluded that bone impairment in Noonan syndrome is likely primary and not secondary to any of the phenotypic traits of the disorder (Baldassarre, 2016).

Cardiac symptoms are the most common initial presentation for patients with RASopathies and patients with congenital heart disease (CHD) are diagnosed much younger than patients without CHD (Jhang 2016). Hypertrophic cardiomyopathy (HCM) is frequently observed in patients with RASopathies, with varied prevalence by diagnosis. The elucidation of the genetic mutations for Noonan syndrome has made clear that the risk for HCM is dependent on genotype. Diagnostic genetic testing can be helpful in determining an individual's risk for HCM. Treatment for cardiomyopathy associated with mutations in *RAF1* may also be possible. MEK inhibitors and HMG-CoA reductase inhibitors are potential avenues for treatment (Gelb 2015).

Mutations that cause Noonan syndrome have been associated with delayed puberty. A case series of 4 patient with Costello or CFC developed precocious puberty, suggesting complex regulation of the hypothalamic–pituitary–gonadal axis and the timing of puberty

by the RAS-MAPK pathway. Additional study of the timing of puberty in this population is warranted (van der Kaay 2016).

Tumor and Cancer Research
Individuals with NF1 have an increased risk for tumor development and therapy with MEK inhibitors have shown a reponse in tumor size in pediatric patients with inoperable plexiform neurofibromas (Dombi 2016). The *BRAF* gene encodes a serine/threonine protein kinase that participate in the MAPK/ERK signaling pathway and plays a vital role in cancers and the RASopathies. *BRAF* has been shown to be associated with CFC, Noonan syndrome, and NSML as well as papillary thyroid tumors, melanomas, colorectal tumors, prostate tumors, and lung cancers. One study confirmed the occurrence of the *BRAF* V600E mutation in Langerhans cell histiocytosis (Hussain 2014; OMIM 164757). *BRAF* and *RAS* mutations have been identified in sporadic and secondary pyogenic granulomas, a common benign vascular skin lesion (Groesser 2016).

In a cohort of 735 individuals with germline mutations in the RAS signaling pathway, there were 12 cases of cancer compared to an expected 1.12 cases based on German population-based incidence rate. This corresponds to a 10.5-fold increased risk of all childhood cancers combined (specifically, juvenile myelomonocytic leukemia, brain tumor, acute lymphoblastic leukemia, rhabdomyosarcoma, and neuroblastoma) (Kratz, 2015).

Somatic mutations in *NF1* occur in 5-10% of human sporadic cancers and may contribute to resistance to therapy (Ratner 2015).

Research Plan:

Study Population:

The study is open to all eligible patients regardless of gender or ethnicity. Minors as well as adults will be enrolled in this study. The parent(s)/legal guardian(s) will provide informed consent for minors. Informed consent will be obtained in the format of the participating institution and in accordance with International Conference on Harmonization (ICH) guidance documents.

The total number of participants is not a targeted number, and there will be no cap on the number of people who can enroll in this study. Initial enrollment of approximately 50 and subsequent new enrollment of approximately 50 patients annually is expected.

Selection and Recruitment of Participants:

There are different ways by which participants may be recruited:

1. Participants may be recruited in CCHMC's primary care or specialty clinics (to include, but not limited to Human Genetics, Hematology/Oncology, Neurology, Developmental and Behavioral Pediatrics (DDBP) or any other clinic or department at CCHMC that cares for individuals with RASopathies).

a. Clinicians at CCHMC may request that we recruit participants under their care after they determine the participant's eligibility and obtain participant permission to forward their contact information to our study team. Alternatively, CCHMC clinicians may provide individuals under their care with our contact information for the family to contact us directly.
b. Eligible participants may be approached for participation when they are scheduled for a clinical visit. Every attempt will be made to obtain specimens at the time of clinically indicated procedures.

2. Eligible participants may be contacted by telephone, mail, mychart, or email.
   a. We may develop recruitment materials (i.e. flyers, social media posts, websites, et cetera) that we will use for the purpose of informing families about the opportunity to enroll in this study. All materials will be submitted to the IRB for approval. We may work with Research Marketing to ensure high quality and appropriate CCHMC branding of all materials.
3. Physicians and any collaborators at sites other than CCHMC who learn of our research study may request that we recruit participants currently under their care. A physician must first determine their participant's eligibility and obtain participant permission before forwarding any contact information to our research team.
4. When research staff are able to attend, eligible patients registered for and/or in attendance at scientific or patient advocate meetings will be invited to participate. Appropriate application and collaboration with the meeting's research staff will occur. Families may be offered the opportunity to enroll prior to, during, or following conferences/events that the study team has been invited to attend, but immediate enrollment will not be required.
   a. RASopathiesNet is one organization that holds meetings for scientists and patients. Their meetings are held in the United States approximately every two years.
5. The study team may perform an electronic medical record query (i.e. slicer dicer) to aide participant recruitment.
6. A participant may be self-referred if they become aware of our research study through our website, any other website, clinicaltrials.gov registration, other participants, conferences/events or any promotional materials (brochures, newsletters, flyers). A self-referred participant may contact the research coordinator or any member of the study team directly to learn more about the study and potentially proceed in the enrollment process.
7. Family members of individuals with a RASopathy will be recruited during/after the index participant recruitment.
8. On rare occasions, medical history and/or specimen from deceased persons with a known or suspected RASopathy, not previously enrolled in this protocol, may be included if permission is obtained from the deceased person's appointee (i.e., parent/guardian, closest living relative, or person with medical power of attorney). This data may be acquired and studied.

Eligible patients who are willing to participate will be invited to submit data to the database and/or specimens to the repository.

Unless a participant requests that his/her samples, study records, and medical information be removed from the repository, samples and medical information will remain in the repository indefinitely.

In addition to conducting research at CCHMC, de-identified samples and/or relevant clinical information may also be sent to outside institutions and/or researchers or for-profit organizations that are conducting relevant research. The outside institution and/or researcher must provide Dr. Weaver and/or her designees with a copy of the IRB approval letter for their study. Clinical information sent to outside institutions or for-profit agencies will be identified with a unique study ID only.

Inclusion Criteria:

1) Patients with a suspected or known diagnosis of any of the group of disorders known as RASopathies (e.g., Neurofibromatosis, Costello Syndrome, Noonan Syndrome). Diagnosis may be made clinically and/or confirmed through genetic testing.
2) Unaffected relatives of patients with a suspected or known diagnosis of any of the group of disorders known as RASopathies.

Exclusion Criteria:

1) Individuals who do not have a suspected or definite diagnosis of a RASopathy.
2) Individuals who do not have a relative with a suspected or definite diagnosis of a RASopathy.
3) Patients who do not have the ability/capacity to undergo the informed consent process OR whose parent/legal guardian is unable to undergo the informed consent process.

Study Procedures and Collection of Samples:

The patient population that is being studied is heterogeneous. Studying this group of disorders necessitates a customized approach to investigation in order to address the unique pathology of each clinical presentation. The procedures expected to be performed may include:

- Thorough review of the patient's medical history including extensive description of the phenotype.
- Genetic studies involving DNA and RNA, such as gene sequencing, microarray analysis, and genotyping.
- The establishment of induced pluripotent cell lines from blood or fibroblast cells obtained via skin biopsy to serve as a source of cellular and genetic material for study.
- Storage of cells, tissues, DNA, and/or RNA for potential study.

- Storage of plasma and/or serum for future currently undefined research related to RASopathies.
- Clinical information and outcomes will be collected from patient evaluations, surveys/assessments, medical intake form(s), and treatment plans.
- Behavior, learning, IQ assessments (ex: Kauffman Brief Intelligence Test, Second Edition KBIT-2; Pediatric Anxiety Rating Scale PARS, DuPaul ADHD rating scale)
- Neurodevelopment surveys (ex: NISONGER, Vineland Adaptive Behavior Scales III, and SRS™-2 Social Responsiveness Scale)
- Collection/use/disclosure of images/video/audio recordings. These records may be used for purposes of study, research, and teaching and may be published in scientific publications. The patient's or family's name may not be used. This release is effective until revoked in writing by the undersigned. Such revocation shall only be effective to prevent any expanded future use of the records.
- Collection and possible storage of blood, skin fibroblasts, left-over tissue from clinically obtained tissue samples.
- De-Identified samples obtained under this protocol may also be sent to institutions outside of CCHMC to investigators or for-profit organizations that are conducting relevant research. Information that could identify subjects will not be released to non-CCHMC investigators.

Bone Marrow Biopsy Collection

Bone marrow will only be collected if a participant is already undergoing a clinically indicated bone marrow biopsy.

Skin Tissue Collection

Skin tissue may be obtained:

a) When the patient is undergoing a clinically indicated dermatologic procedure,
b) When the patient has consented to a skin biopsy for research purposes.
    1) Punch biopsy, shaving, or excision may be used as clinically appropriate, using the appropriate aseptic and anesthetic techniques. The specimen collection will not exceed more than one collection per year unless the collection is being performed as part of clinical management.
    2) Squame adhesive tape disks may be used to collect samples of the stratum corneum. This is a non-invasive collection technique. There is some discomfort when the tape is removed similar to pulling a band-aid off the skin. There are no other risks from this procedure.

Blood, Buccal Cell, Sputum, and Urine Collection

Specimen collection specifically for this biorepository should not exceed more than three collections per year; this will be tracked in the REDCap database. Additional samples may be included in the biorepository if they are collected for

clinical purposes or as part of another research study in which the patient is enrolled.

The collection of blood will be coordinated with clinically indicated procedures whenever possible. No more than 3 ml of blood per kg of body weight will be collected during any single blood draw.

Residual Tissue Specimens

Residual tissue specimens that are normally discarded after a clinically indicated procedure (i.e., tumor excision, bronchoalveolar lavage, etc.) may be collected and stored after every procedure. Identifying information will not be transferred to the storage container, which will only be marked with a unique sample identifier that is not related to any clinical identifiers.

1) **Blood Draw**

   Preferably, patients will be undergoing a clinically indicated blood draw at the time of this procedure; however, we will ask that participants consider donating blood up to a total of three collections per year. The specimen will be processed, frozen, and subsequently stored at CCHMC or processed and frozen at an outside institution and sent to CCHMC.

   Blood volumes will be based on patient weight and estimated blood volume. No more than 5% of the patient's estimated blood volume will be taken for both clinically indicated and research purposes. The first priority will be clinically indicated volumes.

2) **Skin Biopsy**

   The skin biopsy will be processed (including skin fibroblast cultures), frozen, and subsequently stored at CCHMC or processed and frozen at outside institution and sent to CCHMC.

3) **Buccal Cells**

   Samples may be collected using a swab, saliva, or oral-rinse and collected per institutional standards. Samples may be collected for research purposes only or as residual tissue. The cells will be processed, frozen, and subsequently stored at CCHMC or processed and frozen at outside institution and sent to CCHMC.

4) **Sputum**

   Sputum samples will be collected per institutional practice. Samples may be collected for research purposes only or as residual samples. These samples will be processed, frozen, and subsequently stored at CCHMC or processed and frozen at outside institution and sent to CCHMC.

5) **Urine Collection**

Urine samples will be collected per institutional practice. Samples may be collected for research purposes only or as residual samples. These samples will be processed, frozen, and subsequently stored at CCHMC or processed and frozen at outside institution and sent to CCHMC.

6) **Tissue Samples**
A sample of any tissue (i.e., malignancy, hamartoma, plexiform neurofibroma, or any other clinical specimen) which is removed for clinical purposes will be processed fresh or frozen and stored for further research. The specimens will be obtained from the diagnostic biopsy, as available, or from resected tissue if it is removed for therapeutic reasons. The tissue specimen will be processed, frozen, and subsequently stored at CCHMC or processed and frozen at outside institution and sent to CCHMC.

7) **Bone Marrow Aspiration**
Samples will be collected from participants who are undergoing a clinically indicated bone marrow aspirate. A bone marrow aspiration will be conducted per standard procedure and no more than 10 mL of additional bone marrow for research purposes will be aspirated. The sample will be processed, frozen and subsequently stored at CCHMC or processed and frozen at outside institution and sent to CCHMC.

Sample Acquisition and Storage

Samples will be acquired after participants are recruited and consented. For patients undergoing sample collection for clinical purposes, every effort will be made to combine the research sample collection with the clinical collection to minimize risk to the participants. The method of specimen collection will be recorded by the Clinical Research Coordinator (CRC) in the REDCap database.

Samples will be logged and stored in the Cincinnati Biobank.

Research Database

A REDCap database will be used to store the collected information, which will be labeled with a unique sample identifier that is not related to any clinical identifiers. Access to the database will be restricted to the Principal Investigator and his designees, where each designated individual will be assigned a unique User ID/password combination. Specimen and medical history/information will be entered into the database.

Participants may be contacted by phone, mail, e-mail, or in person by a study investigator, coordinator, or designee to request participation in questionnaires/surveys/assessments. Interested study participants may receive surveys/assessments via a printout or electronically (e.g. pdf or REDCap survey link). Completion of electronic questionnaires may take place during study visits using CCHMC resources (tablet, computer, signature pad) currently available to the study team. Alternatively, surveys can be sent to the participants via mail, text, or email or administered over the phone by a member of the study team.

As described earlier, this study is customized by disorder, phenotype, and/or individual participant. For example, the study team may want to compare participants with a particular genetic mutation and/or phenotype. The study team will assess which surveys/biospecimen/assessments are appropriate for each participant. Preferences of each participant, outlined in the consent form, will direct these decisions.

Examples of Database Records:

- Demographic information
- Medical records
- KBIT-2 (Kaufman Brief Intelligence Test, Second Edition)
- Surveys (to be assigned by study PI or designee)
  - Condition/disorder/diagnosis specific medical history questionnaires
    - medical intake form used in a specialty clinic for patients with a confirmed or suspected diagnosis
    - i.e. Smith Kingsmore Medical History
  - i.e. Costello Clinical QuestionnaireChild Behavior Checklist (CBCL)
  - Physical and Neurological Examination of Subtle Signs (PANESS)
  - Social Communication Questionnaire version Lifetime (SCQ-L)
  - Modified Checklist for Autism in toddlers (M-CHAT)
  - Child Health Questionnaire Parent Form (CHQ-PF50)
  - SF-36 Health Survey
  - PedsQL (child self-report and/or parent proxy report)
  - Functional communication classification system (FCCS)
  - Pediatric Inventory for Parents (PIP)
  - Other questionnaires: BRIEF, SRS, Conners-3, Y-BOCS

Release of Specimens and Clinical Data to Other Investigators

Release of fresh or frozen specimens and clinical data to both CCHMC and External investigators must be approved by the Repository Use Committee through the completion of a specimen request form. Among other data, the specimen request form will require information concerning: Principal Investigator, funding sources, a research synopsis, IRB approval of the research project, and details about the required samples. At the time a de-identified sample is requested, the requesting investigator may also request de-identified clinical data if needed.

Completion of the specimen request form and approval by the Repository Use Committee is required before samples will be distributed. The request form specifies that the investigator must not try to re-identify the subjects from whom the samples are derived. Under no circumstance may an investigator provide these samples for use to additional investigators unless specified otherwise in an approved sample request.

The Repository Use Committee is comprised of at least 1 Co-Investigator and/or the Principal Investigator as well as another member of the study team (e.g., CRC) to scientifically evaluate applications and ensure proper distribution of the samples. Other eligible members of the Repository Use Committee include members of the RASopathy

Program Clinical Team. Members of this team meet regularly to review current clinical and research RASopathy topics.

This committee will meet as needed to review sample requests. Projects may also be approved via email. Members will excuse themselves during discussions in the case that they have requested samples. The chair of the committee, with agreement from at least one other member of the committee, will have the authority to approve minor changes to requests (i.e. addition of a small number of samples to an already approved study).

Once the Repository Use Committee approves the scientific/technical merit of a specimen request, the samples will be released to the requesting investigator.

Facilities Utilized in the Study

The specimens will be collected and processed using the facilities of several divisions within Cincinnati Children's Hospital Medical Center, including the Division of Human Genetics and Clinical and Translational Research Center (CTRC). Repository samples with unique identifiers will be processed and securely stored in the Cincinnati Biobank.

POTENTIAL BENEFITS

Participation in this study will benefit science through advancement of knowledge in the diagnosis and treatment of patients with RASopathies. There will be no anticipated direct benefit to the patient.

POTENTIAL RISKS, DISCOMFORTS, INCONVENIENCES AND PRECAUTIONS

Special Considerations

Psychiatric Indication:

Physical and/or emotional stress can occur in this patient population (e.g. related to symptom(s) of the disorder). If a participant discloses any suicide ideation or reveals compulsions to harm him/herself or others, the study staff will contact the participant's mental health provider or PCP and/or Psychiatric Intake Response Center (PIRC) at Cincinnati Children's Hospital for further assessment and guidance. The Pediatric Intake Response Center (PIRC) manages psychiatric patient consultation and intake for Cincinnati Children's Hospital Medical Center. Staff members are available 24 hours a day, seven days a week at 513-636-4124 or psychiatryresponse@cchmc.org .

Radiation Safety: Not applicable
Investigational Drugs and Devices: Not applicable
CCHMC Pharmacy: Not applicable
DSMB: Not applicable
Discarded tissues: De-identified discarded tissues may be used in this study to establish reference ranges or normative comparisons for particular assays or assessments used in this study.
Cell Storage: Blood, marrow, skin tissue and/or residual specimens obtained through this research protocol will be prepared, frozen, and subsequently stored at CCHMC or processed and frozen at outside institutions and sent to CCHMC.

Genetic Studies: The proposed protocol includes genetic studies. These include, but are not limited to, molecular, cytogenetic, and epigenetic analyses. Results of genetic studies will not be returned to participants.

Standard of care will be followed for all procedures performed to decrease the risk of infection, pain, bleeding, and bruising.

There is a small risk that protected health information may be released to an unauthorized individual. Collected data will be stored in a secure database maintained by Cincinnati Children's Hospital Medical Center. Only the Principal Investigator and designees will have access to the secured database. A unique patient number will be assigned to each study subject and corresponding samples. No identifying names will be affixed to the specimens in storage. The identifying key will be securely stored and accessed only by the study team.

We do not anticipate adverse medical events directly associated with this protocol. Adverse events will be monitored by the Principal Investigator and research personnel involved in the conduct of the study and reported according to CCHMC IRB guidelines.

Risk evaluation: Minimal risk but without direct benefit to the participants.

DSMB: This is a minimal risk study and does not require a DSMB. The data generated during this trial will be monitored by the PI for safety and compliance with protocol-specified requirements.

CONFIDENTIALITY

The research study staff will be collecting information from the participant's medical record. This information will be collected by research study staff, and will only be accessible to research study staff of this study, and, when applicable, to research study staff of associated future use research studies. The electronic research data will be password-protected and maintained on CCHMC computers. All non-electronic study data will be maintained by the research study staff in the offices of the Division of Human Genetics. Specimens collected for this research study will be stored with the Cincinnati Biobank. Lab staff in the Cincinnati Biobank may access patient identifiers in tracking specimens. The Cincinnati Biobank will maintain study data in their sample tracking software with restricted permissions to Cincinnati Biobank staff.

A unique study number will be assigned to each study subject and corresponding specimen. The identifying names will be linked through an identifying code to the subject number. Only the PI and designees will have access to the key.

Only the database coordinator will be able to link the identity of the patient to the unique identifier from the specimens. The database coordinator will only release information to the Investigator(s) that is de-identified.

Manuscripts or presentations resulting from this protocol will only identify participants by an arbitrary subject ID number.

PERIOD OF TIME ESTIMATED TO COMPLETE PROJECT AS DESCRIBED

The study will continue indefinitely with no specific end date.

FUNDING

This research study is funded by the Divisions of Human Genetics and Experimental Hematology & Cancer Biology at CCHMC.

PAYMENT FOR STUDIES

There will be no financial compensation for participation in the study.

RESEARCH PROTOCOL, CONSENT FORM, AND CORRESPONDENCE

A current copy of the approved research protocol, consent form, and all correspondence will be maintained by the office of Dr. K. Nicole Weaver (CCHMC).

For outside institutions, the protocol and the informed consent (as applicable) must be reviewed and approved by each institution's IRB before study may be initiated. Written informed consent will be obtained in the format of the participating institution, and in accordance with International Conference on Harmonization (ICH) Guidelines.

A verbal discussion will take place with the participant and/or the participant's parent(s)/legal guardian(s) in person or by telephone that will include an opportunity to ask any questions.

For minor subjects who are brought to CCHMC by a person other than the parent or Legally Authorized Representative (LAR), the consent form may be sent to the parent for review. The consent process will take place over the phone. If the parent or LAR agrees to allow the participant to take part in the study, a copy of the signed consent will be returned and reviewed by a study coordinator before any research procedures occur. The consent form can be returned to CCHMC by fax, e-mail, or regular mail.

For participants who do not speak English, we will utilize the short form consent process. A translator or qualified bilingual staff member will be present for the consent process. The participant will sign both a short form and the full consent document.

A separate consent form will be signed by each participant. Subjects 11 years and older will provide documented assent via a signature on the consent form.

When a subject turns 18, they will be asked to re-consent to their specimens remaining in the repository. If unable to obtain re-consent from a subject within a year after the investigator makes a diligent effort to contact the subject reaching the age of 18, the investigator may retain the data as is in the repository. The only exception is if the subject contacted the team and asked to have samples removed from the repository, then this request would be honored.

No samples or data will be collected and no research will be performed on the specimens until full written consent is obtained from the patient or his/her legally authorized representative.

If a participant should choose to withdraw their consent, any collected samples and/or data that has already been published or analyzed will remain in the database in a de-identified state. No further analysis of the samples for research purposes will be conducted and the samples will be discarded. No additional clinical data will be collected and entered into the database, however already existing clinical information and/or surveys will remain in the database in a de-identified state.

eConsent:

Electronic informed consent will be developed and implemented using REDCap. REDCap is a secure web application for building and managing online surveys and databases. While REDCap can be used to collect virtually any type of data (including 21 CFR Part 22, FISMA, and HIPAA-compliant environments), it is specifically geared to support online or offline data capture for research studies and operations.

The IRB approved consent document will be uploaded into the database instrument. The IRB approved consent will be modified to an electronic format that includes all the same elements found on the paper document. The elements of the consent requiring a signature has been added as a generate field. The instrument includes fields to capture full name, signature, and date and time of the signature for the consenter. When completed REDCap will generate a footer that contains the long date and time the document was submitted and “Confidential” listed in the header as an added precaution to preserve the research participants confidentiality . REDCap's 'Auto-Archiver + eConsent Framework' will be used. The 'Auto-Archiver + e-Consent Framework' survey option adds two things to the typical survey-taking process. 1) Before a participant completes the survey, an extra certification page is added to end of the survey that displays an in-line PDF copy of their survey responses in which they will be asked to confirm that all information in the document is correct. Once they confirm all is correct, the survey will then be marked as complete. The survey will not be considered complete until they fulfill the certification step. 2) Upon completion of the survey, a static copy of their responses in the form of a consent-specific PDF will be stored in the project's File Repository. The consent-specific PDF may have the values of the e-Consent Framework Options inserted at the bottom of each page in the PDF. These values (i.e., name, date of birth, etc.) are added to the PDF as extra documentation of the identity of the person who is consenting.

The HIPAA Consent is queued to automatically open once the consent has been signed and all logic is satisfied.

For the HIPAA and Notice of Privacy Practices the IRB approved consent document will be uploaded into the database instrument. The instrument includes fields to capture full name, signature, and date and time of the signature for the consenter, and witness and

conditional text that states that all signatures are associated with the Subject ID# registered in the database.

Signed and submitted documents will be available as a PDF in REDCap's File Repository. A PDF of the eConsent document will be sent to CCHMC HIM per requirements and long-term storage will be at the CCHMC approved vendor, LabArchives, which is 21CFRPart11 compliant.

MANAGEMENT OF UNANTICIPATED FINDINGS

We will not return unanticipated research findings to participants or their family members. Research results that may be of clinical importance to the participant (based on the assessment of Dr. Weaver or designee) may be returned. If results are to be returned the study team will inform the IRB to set up an appropriate plan.

PROTOCOL RECORDS

All study documents will be kept secure. Access to files will be limited to the Principal Investigator and designees only. Study records will be maintained in the secure database that has restricted access to only the Principal Investigator and designees.

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